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Meeting of the States Parties to the Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on Their Destruction

2018 Meeting
Geneva, 4-7 December 2018

Meeting of Experts on Review of developments in the field of science and technology related to the Convention
Geneva, 9-10 August 2018
Item 7 of the provisional agenda
Genome editing, taking into consideration, as appropriate, the issues identified above

Technical Working Paper on Genome Editing and Other Scientific and Technological Developments of Relevance to the Convention

Submitted by Switzerland

I. Introduction

1. The Biological and Toxin Weapons Convention (BTWC) faces a significant acceleration of relevant scientific and technological developments. This may put into question previous certainties and achievements. As a consequence, there is a vital need to identify relevant developments, consider their implications, and pinpoint any necessary individual or collective action to address possible challenges. A meaningful technical exchange among experts at the MX2 – Review of Developments in the Field of Science and Technology Related to the Convention – will be a much needed first step in that regard. To facilitate an interactive technical debate, this Working Paper puts forward non-exhaustive considerations of scientific and technological developments Switzerland deems relevant for expert discussions in the framework of MX2. It focuses on the specific topic for 2018, i.e. genome editing, paying particular attention to advances in CRIPSR technology[1]. It also covers the issue of nucleic acid origami[2].

II. Genome Editing

2. Genome editing is not a new field of active research and existed long before today's often cited CRISPR technology. Even CRISPR, which is an abbreviation for 'clustered regularly interspaced short palindromic repeat', was already described in the early

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1990s[1]. It took, however, another decade to recognize that these particular regions in bacterial DNA do in fact contain short pieces of foreign genetic material. A 2005 report[3] made the link to a never before described adaptive bacterial immune system, i.e. an immune system that is in a position to adapt to new challenges it is faced with. In 2012, another report[4] elaborated on the mechanistic details of this immunity that relies on Cas (CRISPR-associated) proteins. This study also coined the concept of CRISPR-Cas9 as a tool for making edits to a genome. Shortly thereafter, further studies[5,6] showed the vast array of potential applications of CRISPR technology that allows to edit genomes in a way that significantly outperforms existing tools in terms of simplicity, speed and costs.

3. Since then, CRISPR technology has spread across the globe and is being applied in an ever growing number of fields, including synthetic biology, disease modelling, and in therapeutic approaches at the genetic level. A myriad of reports in the scientific literature have been published in recent years, among other issues on the correction of genetic mutations in viable human embryos[7], or the application of so called 'gene drives' to alter the genome of an entire mosquito population at exponentially faster rates than Mendelian inheritance would suggest[8]. These also gave rise to concerns in terms of safety and ethics[9].

4. Not preempting any future groundbreaking discoveries related to the still nascent CRISPR technology, it may likely boil down to the same well-trodden path of previous novel technologies: In various settings, its applicability proves more difficult than anticipated and solutions to any encountered obstacles demand an ever more complex degree of sophistication. For instance, longer-term studies of 'gene drives' have uncovered the emergence of resistance[10,11] that will add much complexity to any future attempts aimed at making it a useful tool in practical terms. Furthermore, a proof of concept study on the potential applicability of CRISPR technology in gene therapy[12] highlighted problems with efficiency of delivery and so called 'off-target effects'. Since improvements in these areas are of utmost importance for future success, several reports have been published recently on related advances: increasing delivery rates by virus-mediation[13], lowering off-target effects by increasing the fidelity of Cas9[14], and the description of an increasing spectrum of alternate proteins[15] such as Cpf1 and Cas13a.

5. Despite such promising advances that try to level out encountered difficulties, the root cause of some of the problems, such as the efficiency of CRISPR technology in particular cell types, may be more intricate than anticipated. For instance, results of a mechanistic analysis in human pluripotent cells demonstrated that reduced efficiencies of CRISPR technology seem to be based on inhibition by p53[16,17] – short for cellular tumor antigen p53 –, a protein sometimes labelled as 'guardian of the genome' alluding to its protective function as a tumor suppressor by preventing mutations and thus preserving genome stability. In other words, it appears that CRISPR technology works in cells that may have inactive or corrupt copies of p53. This could lead to selection of cells in which CRISPR technology works but at the cost of increased genome instability which, in turn, could lead to cancer[18].

6. In yet another area of active research, CRISPR technology, on the one hand, was commented as potentially obfuscating current microbial analysis approaches of value to forensics. This challenge emanates from the mode of action of CRISPR technology that hardly leaves any 'traces', unlike any former techniques would do. Hence, CRISPR technology inevitably decreases confidence in differentiation between genetic engineering and conventional mutagenesis[9], and likely between naturally occurring and man-made alterations. CRISPR technology may, on the other hand, open up new avenues for rapid, inexpensive, sensitive, and potentially field deployable nucleic acid detection through CRISPR-Cas13a[19].

III. Other Scientific and Technological Developments

7. The ability to fold nucleic acid strands into designed two- or three-dimensional shapes and structures, sometimes with a 'built-in' ability to perform specific mechanical functions and movements, exists already for many years[2]. Until recently, such nucleic acid origami encompassed a cumbersome design process through the creation of purified DNA strands. This niche technology suffered from an important bottleneck caused by a laborious and expensive nucleic acid synthesis, which prevented any serious up-scaling ambitions. A first significant step was achieved by either tapping into naturally occurring DNA[20], especially from abundantly available viruses, serving as 'scaffold', or by using a system of nucleic acid 'bricks'[21,22] for the customized building of shapes and structures. Less than a year ago, scientific reports demonstrated that biotechnological mass production was possible for programmable self-assembly of ever larger structures[23,24,25,26].

8. With the increased accessibility of nucleic acid origami, structures able to perform specific mechanical functions and movements may gain renewed attention from the scientific community and other stakeholders. Especially systems designed to carry molecular payloads[27,28] may now become more relevant for discussions on advances in science and technology within the BTWC.

IV. Conclusions and Recommendations

9. CRISPR technology can reasonably be expected to surprise us with new twists and turns impossible to predict. This is likely also true for many other areas, including the above discussed nucleic acid origami, but also for synthetic biology or the neurosciences. In this context, it will be important to keep track of scientific and technological advances, and their potential bearings on the Convention. At the upcoming MX2, we should hold a technical discussion on genome editing technologies, and especially CRISPR technology, to then take the next step towards an assessment of their implications for the Convention by putting them into the broader context of the 'new era of biology'. In doing so, we should broaden our traditional focus of 'pathogens causing disease' to the wider prospects and implications that developments in the biosciences as a whole may have. Furthermore, technical discussions should also take into account any intangible aspects (e.g. 'tacit knowledge') of advances in science and technology, which may significantly shift initial perceptions about benefits and risks. All of this will allow for a holistic and more realistic understanding of the benefits and risks to the Convention.

V. References

[1] Invernizzi C. CRISPR and the Hype Cycle. Defence Future Technologies – What we see on the horizon (Publisher: armasuisse W+T). 2017 Nov 15;81-3.

[2] Lentzos F & Invernizzi C. DNA Origami: Unfolding Risk?. Bulletin of the Atomic Scientist – Analysis. 2018 Jan 25.

[3] Mojica FJ et al. Intervening sequences of regularly spaced prokaryotic repeats derive from foreign genetic elements. J Mol Evol. 2005 Feb;60(2):174-82.

[4] Jinek M et al. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science. 2012 Aug 17;337(6096):816-21.

[5] Cong L et al. Multiplex genome engineering using CRISPR/Cas systems. Science. 2013 Feb 15;339(6121):819-23.

[6] Mali P et al. RNA-guided human genome engineering via Cas9. Science. 2013 Feb 15;339(6121):823-6.

[7] Tang L et al. CRISPR/Cas9-mediated gene editing in human zygotes using Cas9 protein. Mol Genet Genomics. 2017 Jun;292(3):525-33.

[8] Hammond A et al. A CRISPR-Cas9 gene drive system targeting female reproduction in the malaria mosquito vector Anopheles gambiae. Nat Biotechnol. 2016 Jan;34(1):78-83.

[9] Ledford H. CRISPR, the disruptor. Nature. 2015 Jun 4; 522(7554): 20-4.

[10] Unckless RL et al. Evolution of Resistance Against CRISPR/Cas9 Gene Drive. Genetics. 2017 Feb;205(2):827-41.

[11] Champer J et al. Novel CRISPR/Cas9 gene drive constructs reveal insights into mechanisms of resistance allele formation and drive efficiency in genetically diverse populations. PLoS Genet. 2017 Jul 20;13(7):e1006796.

[12] Yin H et al. Genome editing with Cas9 in adult mice corrects a disease mutation and phenotype. Nat Biotechnol. 2014 Jun;32(6):551-3.

[13] Maddalo D et al. In vivo engineering of oncogenic chromosomal rearrangements with the CRISPR/Cas9 system. Nature. 2014 Dec 18;516(7531):423-7.

[14] Kleinstiver BP et al. High-fidelity CRISPR-Cas9 nucleases with no detectable genome-wide off-target effects. Nature. 2016 Jan 28;529(7587):490-5.

[15] Zetsche B et al. Cpf1 is a single RNA-guided endonuclease of a class 2 CRISPR-Cas system. Cell. 2015 Oct 22;163(3):759-71.

[16] Haapaniemi E et al. CRISPR-Cas9 genome editing induces a p53-mediated DNA damage response. Nat Med. 2018 Jun 11. doi: 10.1038/s41591-018-0049-z. [Epub ahead of print]

[17] Ihry RJ et al. p53 inhibits CRISPR-Cas9 engineering in human pluripotent stem cells. Nat Med. 2018 Jun 11. doi: 10.1038/s41591-018-0050-6. [Epub ahead of print]

[18] Houser K. CRISPR Could Help Us Cure Diseases; It Could Also Cause Cancer. Futurism. 2018 Jun 11.

[19] Gootenberg JS et al. Nucleic acid detection with CRISPR-Cas13a/C2c2. Science. 2017 Apr 28;356(6336):438-442.

[20] Rothemund PWK. Folding DNA to create nanoscale shapes and patterns. Nature. 2006 Mar 16;440(7082):297-302.

[21] Wei B et al. Complex shapes self-assembled from single-stranded DNA tiles. Nature. 2012 May 30;485(7400):623-6.

[22] Ke Y et al. Three-dimensional structures self-assembled from DNA bricks. Science. 2012 Nov 30;338(6111):1177-83.

[23] Tikhomirov G et al. Fractal assembly of micrometre-scale DNA origami arrays with arbitrary patterns. Nature. 2017 Dec 6;552(7683):67-71.

[24] Ong LL et al. Programmable self-assembly of three-dimensional nanostructures from 10,000 unique components. Nature. 2017 Dec 6;552(7683):72-77.

[25] Wagenbauer KF et al. Gigadalton-scale shape-programmable DNA assemblies. Nature. 2017 Dec 6;552(7683):78-83.

[26] Praetorius F et al. Biotechnological mass production of DNA origami. Nature. 2017 Dec 6;552(7683):84-87.

[27] Andersen ES et al. Self-assembly of a nanoscale DNA box with a controllable lid. Nature. 2009 May 7;459(7243):73-6.

[28] Douglas SM et al. A logic-gated nanorobot for targeted transport of molecular payloads. Science. 2012 Feb 17;335(6070):831-4.